THE VENOUS OXYGEN CONTENT AND THE ALKALINE RESERVE OF THE BLOOD IN PNEUMONIC INFLUENZA.

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The skin of persons suffering from pneumonic influenza often assumes a distinctly bluish or plum-coloured tinge. This colouration was at first described as a cyanosis, and was attributed to the most likely cause of such a condition: a deficient oxygenation of the blood in the lungs. Such an assumption is the most natural one to make to explain a condition of this kind accompanying a disease of the respiratory system. It was observed, however, that many of the persons whose skins became coloured in this way did not show any marked signs of respiratory distress.

The question therefore arose, whether this colouring of the skin really was a cyanosis in the generally accepted sense of the word, that is to say, whether the colour actually was due to an abnormally large proportion of reduced hæmoglobin in the blood. An excess of hæmoglobin would be the result of deficient oxygenation of the blood, and under ordinary conditions of acidity, would lead to marked dyspnœa.

To explain the absence of obvious respiratory distress in the case of the "cyanosis" accompanying pneumonic influenza several hypotheses have been put forward. It has been suggested that the nerve centre governing respiration may be poisoned; that its sensitiveness may thus be dulled, and that the accumulation of products due to insufficient oxidation may no longer produce its customary response in increased ventilation of the lungs.

Another suggestion is that the colouring matter of the blood may itself be altered somehow. It may no longer be capable of taking up its normal amount of oxygen in the lungs, and a

condition of anoxaemia which Haldane (1918) has described may result. Such a condition might be produced by a conversion of the oxyhæmoglobin of the blood into its isomer methæmoglobin. If in addition, there were decreased sensitiveness of the respiratory centre, such a condition might give rise to an appearance resembling cyanosis, but to no dyspnœa.

A third suggestion is that the colouration of the skin of influenza patients has nothing to do with the degree of oxygenation of the blood, but that it is a direct pigmentation. In this case, no respiratory disturbance would accompany the appearance of the colour.

If, as is supposed in the first hypothesis, the so-called cyanosis is due to deficient aeration of the blood, then the oxygen content of the arterial blood, and also of the venous blood, will be lower than normal, provided that the rate of the circulation does not increase out of all proportion to the metabolism. The amount of carbonic and other acids in this blood, on the other hand, will be abnormally high. Such blood will possess a normal capacity for oxygen; its degree of saturation with oxygen, however, will be abnormally low. The reaction or hydrogen ion concentration of this blood will probably also be normal, since only an extreme degree of acidosis affects the reaction. The reactivity of this blood, however, or its power of neutralising added acid will be abnormally low.

On the second hypothesis the oxygen capacity of the blood as well as its oxygen content will be altered. A given amount of hæmoglobin will not be able to take up its usual charge of oxygen. The reactivity of this blood toward alkali will also be diminished owing to the accumulation of acid products of metabolism.

On the third hypothesis the blood will differ neither as regards its degree of oxygen saturation nor its oxygen capacity from normal, and its reactivity toward alkali will also lie within normal limits.

An investigation which has been carried out by Harrop (1919) supports this last hypothesis. This worker found that the venous oxygen content and the oxygen capacity of the blood of influenza patients remained normal until the final stages of the pneumonic complications were reached. The oxygen capacity and the degree of saturation then began to diminish, and some cases reached very low values before death took place. Harrop

concluded that the apparent cyanosis observed was really a pigmentation of the skin, and was no indication of a disturbed respiratory function during the earlier stages of the disease.

In the present investigation, determinations have been made of the oxygen capacity and the degree of oxygen saturation of the venous blood of persons suffering from pneumonic influenza. In some of the specimens the acidity and the reactivity were determined by means of the hydrogen electrode. The presence of bands due to pigments other than oxyhæmoglobin was looked for in the absorption spectrum of the blood, but the method adopted was not found to be sensitive enough to detect the presence of small quantities of abnormal pigments in the presence of a large proportion of oxyhæmoglobin.

If the determinations of oxygen content could have been made upon arterial instead of venous blood, the results would doubtless have been of greater value. Venous blood passes through the capillary circulation after leaving the lungs, and before it reaches the veins undergoes changes, the extent of which must remain largely a matter of assumption. And these considerations must be of greater weight in conditions in which there is an enhanced metabolism due to fever. With all these reservations, however, it seems clear that, under similar pathological conditions, pronounced variations of the oxygen content of venous blood must be associated with variations in the same direction of the oxygen content of the arterial blood, and some recent work by Stadie (1919) has shown this to be the case. Further, there is the practical aspect of the matter; the obtaining of arterial blood is an operation likely to cause considerable discomfort to a person who is already seriously ill, while the obtaining of a sample of venous blood requires only a few moments, and subjects the patient to a minimum of inconvenience.

The above reservations with regard to venous blood do not hold for the determinations of acidity and reactivity. The methods adopted in the present investigation aimed at the determination of decrease of available alkali or of excess of acids other than carbonic acid, and as this acid is the only acid whose concentration is altered by the conversion of venous into arterial blood, it is a matter of indifference whether the remaining acids are determined in the blood before or after arterialisation.

The samples of blood were collected in the manner described

by Cullen (1917). The blood was taken from a vein in the arm with a hypodermic syringe. The syringe was first washed with liquid paraffin to hinder clotting during the withdrawal of the sample. The sample was then ejected under a layer of liquid paraffin on to a quantity of solid, finely ground potassium oxalate equal to about 0.4 per cent. of the amount of blood taken. The blood was then gently agitated to dissolve the potassium oxalate. In this way the samples are obtained in an unclotted condition without the addition of a liquid, which would alter their dilution, and are exposed to a minimum of risk of coming into contact with the air. It was sometimes necessary to compress the arms of the subjects to render the veins turgid and to facilitate the insertion of the needle, but the compression was removed, and the circulation allowed to resume its course before the sample was withdrawn. The cases of pneumonic influenza from which the samples of blood were obtained were all very serious, and many of them terminated fatally; all showed distinct colouration of the skin.

The following are particulars of cases from which samples of blood were obtained:—

2. A. S., male, 26, single, bricklayer. Admitted 2/4/19, died 18/4/19. Sample taken 16/4/19 (22nd day of disease), temperature 97.7°, pulse 110, respiration 40.

4. D. M. B., male, 21, single, collector. Admitted 16/4/19, died 22/4/19. Sample taken 22/4/19 (8th day of disease), temperature 103°, pulse 136, respiration 36.

6. H. F., female, 11, scholar. Admitted 16/4/19, discharged 1/5/19. Sample taken 25/4/19 (10th day of disease), temperature 98.1°, pulse 92, respiration 25.

7. W. R., male, 24, single, labourer. Admitted 26/4/19, died 6/5/19. Sample taken 1/5/19 (7th day of disease), temperature 102.8°, pulse 84, respiration 25.

8. P. U., male, 24, single. Admitted 26/4/19, discharged 10/5/19. Sample taken 2/5/19, (9th day of disease), temperature 98.2°, pulse 77, respiration 29.

9. A. C., male, 29, single, fitter. Admitted 2/5/19, discharged 30/5/19. Sample taken 5/5/19 (11th day of disease), temperature 98.6°, pulse 90, respiration 26.

11. H. B., male, 27, married, carpenter. Admitted 6/5/19, discharged 16/5/19. Sample taken 12/5/19 (11th day of disease), temperature 99.3°, pulse 70, respiration 24.

The particulars of cases 1 ,3, 5, and 10 were not obtainable.

The oxygen capacity and oxygen saturation of the samples of blood were determined by Haldane's (1897) ferricyanide method in Barcroft's (1908) differential apparatus. The principle of the method is as follows: Blood is laked with dilute ammonium hydroxide. Its oxygen is then liberated by the addition of potassium ferricyanide. These operations are performed in closed vessels connected with a clove oil manometer. The difference of pressure observed in the manometer multiplied by the constant of the instrument gives the volume of oxygen liberated. The volume is then corrected for temperature, barometric pressure, and moisture, the final volume given being that of the dry gas at normal temperature and pressure. In the case of unsaturated blood, oxygen is absorbed during the process of laking, and the negative pressure produced in the manometer is similarly a measure of the volume of oxygen absorbed.

For samples 1—6 Barcroft's small apparatus, which takes 0.1 cc. of blood was used; for samples 7—11 the large apparatus, which requires 1.0 cc. of blood was used. The small apparatus was calibrated by liberating in it the oxygen from a known volume of standard hydrogen peroxide (Barcroft and Burn, 1913). The large apparatus was calibrated by measuring the diameters of the limbs of the manometer and the volumes of the vessels in which the gas was liberated (Barcroft and Higgins, 1911), the constants so found being increased by 2 % to allow for the difference existing between this and the former method of calibration.

The sample of blood for the determination of oxygen capacity was saturated with oxygen by spreading 2 cc. in a thin layer over the inner surface of a 200 cc. flask. The flask was corked to prevent evaporation of the blood, and was kept rotating so as to expose the greatest surface to the air for about 15 minutes. Such blood took up no more oxygen on being laked in the differential apparatus. The samples for the determination of oxygen content were cautiously introduced into the apparatus under a layer of the dilute ammonium hydroxide used for laking, and did not come into contact with the air during the transference except at the narrow upper meniscus of the pipette, and momentarily at its tip. As the pipette was not completely emptied in delivering the sample, the upper portion of the sample, which came into contact with the air was not used in the analysis. The pipettes used were calibrated by weighing

the amounts of blood delivered by them. Care was taken, by thorough mixing, to ensure a uniform distribution of the corpuscles in a sample of blood before withdrawing a portion for analysis.

The percentages of oxyhæmoglobin were determined in two ways: (1) By calculation from the oxygen capacity, taking as 100 per cent. the amount of hæmoglobin in blood which combines with 18.5 volumes of oxygen (at normal temperature and pressure) per 100 cc.; (2) colorimetrically, by comparison of solutions of known dilution with standard tinted glass in the Miescher—von Fleischl haemoglobinometer. The scale of the hæmoglobinometer was calibrated by determination of the oxygen capacity of samples of blood corresponding to different readings. Five series of determinations showed a maximum range of variation of 5 per cent. from the values obtained by the chemical method for a dilution of blood of 1 in 200.

Attempts were made to detect the presence of pigments other than oxyhæmoglobin by examination of the absorption spectrum of the blood. Methæmoglobin was especially looked for. It was not found possible, however, to detect the small proportions of this pigment in the presence of a large proportion of oxyhæmoglobin by means of the spectroscope, as already stated. The effect of various proportions of methæmoglobin on the colorimetric estimation of hæmoglobin in the blood was then determined. It was found that the presence of 5 per cent. of methæmoglobin could be detected with certainty by this method. For a particular sample of blood, this proportion of methæmoglobin altered the reading of the hæmoglobinometer from 40.8 to 30.3, that is, caused an error of 25 per cent in the determination of hæmoglobin, and gave rise to an appreciable change in the tint of the diluted blood. Percentages of methæmoglobin well within the range of variation between the results of the chemical and colorimetric methods of determining hæmoglobin, would therefore be detected by this means.

The acidity and reactivity of the samples of blood were determined by the method described by Cullen *(loc. cit.)*. The corpuscles of the blood were separated from the plasma in a centrifuge. The dissolved carbon dioxide was then removed from the plasma by exposing it in a thin layer for several minutes to a pressure of about 6 cm. of Hg. In this way, uncertainties due to the escape of varying amounts of carbon dioxide during

the measurement were avoided. The electromotive force of a cell made up of a hydrogen electrode dipping into this plasma and a decinormal calomel electrode was then measured as described by Michaelis (1911). The plasma was connected with the calomel electrode by means of a siphon containing saturated ammonium nitrate. A capillary electrometer was used as the null instrument; the readings were made on a Wolff's potentiometer to the nearest millivolt. The acidity or concentration of hydrogen ions (C_H) is calculated from the observed electromotive force by the equation:

$$\log \frac{1}{C_H} = \text{pH} = (\text{E}-0.337)/\text{k}.$$

where E is the observed E.M.F., and k a factor which varies slightly with the temperature.

The reactivity of the plasma towards acid may be determined in two ways: (1) by measuring the amount of acid required to alter the reaction by an arbitrary extent, or (2) by measuring the change of reaction produced by an arbitrary amount of acid. In practice the latter method is much the simpler and more convenient. Cullen *(loc. cit.)* showed that it was convenient to add an amount of acid sufficient to liberate all the combined carbon dioxide in the plasma, the liberated gas being got rid of by exposure to a reduced pressure, as described above. In the present work, the change of acidity produced by adding 2 volumes of 0.0196N HCl was measured after pumping off the liberated carbon dioxide.

In the following table are shown the oxygen content and capacity of a number of samples of venous blood from cases of pneumonic influenza. The percentages of hæmoglobin as determined colorimetrically in the von Fleischl hæmoglobinometer, and as calculated from the oxygen capacity, are also shown.

TABLE I.

Oxygen capacity, oxygen content, and percentage of hæmoglobin of venous blood in pneumonic influenza.

Sample.	Oxygen capacity.	Oxygen content.	Per cent. Chem.	Hæmoglobin Colorim.
1	13·25	71·7	71·5	86·5
2	19·7	68·4	106·5	96
3	20·2	37·9	109	112
4	23·2	72·2	125	125
5	20·8	62·0	105	104
6	17·7	47·9	95·5	109
7	21·7	82·7	117	116
8	19·2	50·8	104	108
9	21·2	57·7	112	110
10	16·4	82·9	88·5	100
11	18·9	38.7	102	99
Average	19·1	61·2	103	106

The above table shows that there is considerable variation, both in the oxygen content and the oxygen capacity of these samples. The oxygen capacity varies between 13.25 and 23.2 cc. of oxygen, at 0°C. and 760 mm. Hg, per 100 cc. of blood, The corresponding percentages of oxyhæmoglobin are 71.5 and 125. These values, therefore, vary over a range of about 25 % of the average, but it is doubtful whether any significance can be attached to this degree of variation. Even in normal individuals the number of red corpuscles in the blood varies over a range at least as wide as this (from 3.5 to 6.0 million per cubic mm.). From the figures available as to the normal range over which the percentage of oxyhæmoglobin or oxygen capacity of blood varies, but there is no reason to doubt that it is approximately the same as that of the number of red corpuscles.

The percentage saturation of the samples with oxygen varies over a slightly wider range, from 37.9 % to 82.9 %. Here again, however, the figures show no general tendency to which any significance may be attached; there is certainly no tendency for the percentage saturation to be low. As many figures lie above the average, which has the normal value, as lie below it And although 60 % is taken as the average normal oxygen saturation of venous blood, this number is likely to be subject to even greater variations than that of the oxygen capacity. Under conditions of disease, in which the metabolism is abnormal, these variations may be still greater, without introducing the necessity

of postulating any alterations of the aeration of the blood itself. The few figures available for the oxygen saturation of normal venous blood confirm this supposition [Twort and Hill (1915), Lundsgaard (1918)]. No conclusions, therefore, can be drawn from a variation of the oxygen content of 30 % in either direction from the normal average.

The figures in this table, therefore, give no evidence of any general decrease, either in the oxygen capacity or in the oxygen content of the blood in pneumonic influenza. The averages of the values given are within 5 % of the averages of normal figures: oxygen capacity, 19.1 % (normal 18.5 %); oxygen saturation, 61.2 % (normal 60 %); percentage of oxyhæmoglobin, 103 (normal 100).

It will be noticed that, although the majority of the values obtained for the percentages of oxyhæmoglobin by the colorimetric method agree well with those calculated from the oxygen capacity of the blood, certain of the results show differences greater than the error of the method (5 %). The results obtained by the mixtures of small percentages of methæmoglobin show that deviations of the magnitude observed might be due to the presence of very small amounts of abnormal pigments, amounts considerably lower than 5 %. Such amounts could not be detected by variations of the oxygen capacity, as the normal variations of this, as has been mentioned, are greater than the variations under consideration. Until further information is obtained however, these variations cannot be definitely attributed to the presence of abnormal pigments in the blood.

The following table shows the concentration of hydrogen ions in the CO_2 free plasma of venous blood from cases of pneumonic influenza, before and after the addition of twice their volume of 0.0196N HCl. The concentrations are expressed both as equivalents of acid per litre (C_H), and in Sörensen's notation in which $pH = \frac{1}{\log C_H}$. The difference of pH and the corresponding increase of C_H, i.e. $\left(\frac{C''_H}{7} - \frac{C_H}{7}\right)$, due to the addition of the acid are also shown. The figures in the columns headed E.M.F. are the values in volts of the potential differences between the plasma and the hydrogen electrode. They are obtained by subtracting 0.377 volts, the potential of the 0.1N calomel electrode, from the observed potential.

TABLE II.

Change of reaction produced by addition of 2 volumes of 0·0196N HCl to 1 volume of CO_2 free oxalate plasma from blood in pneumonic influenza.

Sample	Before Acid.			After Acid.			Diff. pH	$\frac{C''_H}{C'_H}$
	E.M.F.	pH	C'_H	E.M.F.	pH	C''_H		
			10^{-9}X			10^{-6}X		10^{3}X
1	0·418	8·25	5·62	0·294	5·04	9·10	3·21	1·62
2	0·520	8·92	1·20	0·317	5·44	3·63	3·48	3·02
3	0.503	8·62	2·40	0·286	4·90	12·6	3·72	5·25
4	0·521	8·93	1·17	0·289	4·96	11·0	3·97	9·40
5	0·465	7·98	10·5	0·292	5·01	9·77	2·97	0·93
Mean		8·54	3·47		5·09	7·66	3·45	4·04

The above figures show that in the samples examined the acidity of the CO_2 free oxalate plasma ranged from pH 8.93 to pH 7.98 (1.17×10^{-9} N to 10.5×10^{-9}N). After the addition of HCl, the acidity ranged from pH 5.44 to pH 4.90 (3.63×10^{-6} N to 12.6×10^{-6} N). The change of reaction caused by the addition of the acid varied from pH 3.97 to pH 2.97, that is, the acidity was increased from approximately 1000 to 10,000 times by the addition of the hydrochloric acid. The averages of the values for pH in the table before and after the addition of acid are 8.54 and 5.09 respectively. The variations of the individual results from these averages lie between the limits within which the results for normal plasma vary according to the figures given by Cullen *(loc. cit.)*. These figures, therefore, show no abnormalities from which any conclusion may be drawn. In particular, they give no indication of a decreased alkaline reserve; the degree of acidity produced by the addition of a given amount of hydrochloric acid is not definitely greater than would be produced in normal plasma, and no evidence exists for the presence of acid products of intermediate metabolism (acidosis) which would be the result of a deficient oxygenation of the blood. These results are what would be expected from the values obtained for the oxygen capacity and saturation of these samples of blood. Here again, however, attention must be called to the paucity of normal figures for comparison.

Summary.

Samples of venous blood from cases of pneumonic influenza showed no indication of decreased oxygen capacity nor of deficient oxygenation.

The concentration of hydrogen ion produced by the addition of a measured quantity of acid showed no indication of acidosis: the alkaline reserve was not reduced.

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